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Poster Session IV

Enterobacteriaceae: resistance and fitness

NOVEL TIGECYCLINE RESISTANCE MECHANISMS: THE ALTERATION OF THE 30S RIBOSOMAL PROTEIN S10 IN THE DRUG TARGET SITE, IN KPC-2-PRODUCING KLEBSIELLA PNEUMONIAE CLINICAL ISOLATES

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Objectives:

Our recent study performed by comparative genomics of 5 KPC-producing ST512 *K. pneumoniae* clinical isolates, demonstrated that tigecycline resistance can be associated with the increased expression of the AcrAB efflux system, due to depletion of the RamR negative regulator or by the structural alteration of the 30S ribosomal protein S10 in the drug target site, a novel mechanism of resistance identified in one non-RamR mutant (Villa et al., 2013). A screening on a large collection of tigecycline susceptible and resistant *K. pneumoniae* strains of different STs, positive or negative for KPC, isolated in 10 different hospitals in Rome, has been performed in order to better describe the prevalence of RamR and S10 resistance mechanisms and their phenotypes.

Methods:

A collection of 229 *K. pneumoniae* strains, 22 of them showing tigecycline resistance (MIC >2 mg/L) were screened for S10 and RamR mutations. Tigecycline, minocycline and tetracycline MICs were determined by E-test (BioMerieux, IT), disk diffusion and microdilution assays, following procedures and interpreting results as recommended by the EUCAST V 3.0 guidelines. Differential effects of the efflux inhibitor phenyl-arginine-beta-naphthylamide (PAbetaN), Ca²⁺/Mg²⁺ ions and EDTA were evaluated on the RamR and S10 mutant strains.

Results:

All susceptible strains showed conserved RamR and S10 proteins. Among the 22 tigecycline resistant strains, 2 strains showed the mutation Val57?Leu57 in S10 and 10 strains showed the premature interruption of translation of RamR by the insertion of the IS*Kpn18* element or by frameshift mutations. Different mutations and integration sites of the IS*Kpn18* element were detected in the *ramR* gene sequence in the different strains analyzed, demonstrating that independent genetic events occurred in these strains and there was not the spread of a single tigecycline resistant clone among different patients. Interestingly, one strain showed both S10 mutation and RamR depletion. The mechanisms of tigecycline resistance in 10 resistant strains was not identified.

Isogenic S10 and RamR mutants showed comparable MICs for tigecycline/tetracycline (MICs=5/32 mg/L) but S10 showed lower MICs for minocycline (MIC=16 mg/L) than the RamR mutants (MIC=48 mg/L). These differences were confirmed by repeated microdilution and disk diffusion experiments.

Conclusions

Our data suggested that the inactivation of the *ramR* gene was the most common mechanism of tigecycline resistance in our collection. However, the single point mutation in the S10 protein at the tigecycline target-site is also an alternative mechanism of resistance. The mutation in S10 maps in the vertex of a loop that is located at 8 Å from the tigecycline binding site, likely affecting the binding of tigecycline to the 16S rRNA. The phenotype of this mutant was comparable with that conferred by the alteration of the AcrAB efflux pump, but also demonstrated different efficacy toward the three cyclines tested.